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Synthesis and characterization of calcium deficient apatite granules for drug eluting bone graft applications

Mariam Mir[a], Saadat Anwar Siddiqi[a], Tousif Hussain[c], Aqif Anwar Chaudhry[a], Ihtesham Ur Rehman[b], Abdul Samad Khan[a], Ghulam Abbas[a,*]

[a]*Interdisciplinary Research Center in Biomedical Materials, COMSATS Institute of Information Technology, Defence Road, Off Raiwind Road, Lahore, Pakistan*
[b]*Department of Material Science and Engineering, The Kroto Research Institute, University of Sheffield, Sheffield, United Kingdom*
[c]*Centre for Advance Studies in Physics, G.C. University Lahore, Pakistan*

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Abstract

Antibiotic loaded calcium deficient apatite (CDA) granules were prepared by wet chemical synthesis followed by microwave irradiation. Microporous CDA granules were loaded with three broad spectrum antibiotics (Amoxicillin, Gentamicin and Chloramphenicol). The aim of this study was to evaluate the *in-vitro* performance of locally released antibiotics which has a potential to treat infected osseous defects. *In-vitro* studies of the synthesized material elucidated high drug loading capacity and a drug release profile spanning a period of more than 24 h. The respective antibiotic functional groups were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR). X-ray Diffraction (XRD) was used to study the phase purity of synthesized CDA. Drug release profiles were determined by using UV–vis Spectroscopy. Scanning Electron Microscopy (SEM) was used to investigate granule sizes and porosity. Particle size (2–20 μm) with an average pore diameter of 2.9 μm ($\pm$0.5 μm) was achieved without heat-treatment and use of any porogens. Thermal stability was determined using simultaneous Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). The drug release profile showed initial burst release followed by sustained release periodically.

Keywords: Calcium deficient apatite; Local antibiotic therapy; Microporous granules

1. Introduction

Bioactive ceramics including calcium sulfate ($CaSO_4 \cdot 2H_2O$) [1], calcium carbonate ($CaCO_3$, in aragonite form) [2] and calcium phosphates ($Ca(PO_4)_2$) [3] have been used for bone regeneration. Among these, calcium phosphate group (Hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$]), β-tricalcium phosphate [β-TCP, $Ca_3(PO_4)_2$] and calcium deficient apatites (CDA) are suitable candidates for injectable bone substitutes [4], coatings on metallic substrates [5,6] and as dental restorative materials [7]. The chemical composition and structure of calcium deficient apatites and their temperature dependent structural transformation to other apatites like β-TCP have been explored in detail [8]. CDA with a Ca/P molar ratio of 1.5 is structurally similar to HA and stochiometrically to β-TCP [9] and has excellent potential to be used as bone substitute because of its similarity to bone mineral [10]. *In-vivo* studies on CDA have shown that it promotes osteoblast cells colonization and adhesion [11], induces deposition of calcium by cells [12], and considered as a good candidate for tissue regeneration applications. Based on these characteristics there is an increase in application of CDA materials as drug delivery carriers [13,14], minimally invasive surgery [15], as well as orthopedic and maxillo-facial surgeries [16,17].

*Corresponding author. Tel.: +92 42 111 001 007x829; fax: +92 42 35321090.
E-mail address: abbas191@gmail.com (G. Abbas).

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CDA has been synthesized by hydrolysis techniques [18], hydrothermal homogenous precipitation [19,20], continuous hydrothermal synthesis [21] and seed assisted precipitation techniques [22]. A microwave irradiation technique with shorter heating time (3 min) has been reported; however, these experiments resorted to drying the material at 80 °C for 24 h after microwave treatment. The formation of porous calcium phosphate with the aid of polymer mold casting, polymeric slurry addition and sintering methods has been already reported [23–25]. Moreover, other methods including consolidation, isostatic compression or drug adsorption have been used to synthesize antibiotic releasing calcium phosphate. However, the synthesis of porous calcium deficient apatite has been least explored [26,27]. Therefore, it is desirable to synthesize porous bone substitute materials especially those having an average pore size ≤ 10 μm, which have a direct and positive correlation with osteointegration and bone regeneration [28]. The porosity of CDA is an important prerequisite as a drug carrying synthetic bone graft.

Herein, we report the development of a novel CDA antibiotic drug delivery system for potential use in the treatment of infection prone osseous defects. Microporous CDA were obtained by wet chemical synthesis followed by microwave irradiation treatment and loaded with three types of broad spectrum antibiotic including Penicillin (Amoxicillin), Aminoglycoside (Gentamicin) and Protein synthesis Inhibitor (Chloramphenicol). The obtained materials were structurally, thermally and physically analyzed; furthermore, subsequent drug release profiles were also studied.

2. Materials and methods

2.1. Preparation of free and drug loaded CDA granules

The chemicals used in this study were of analytical grade. Calcium Hydroxide [$Ca(OH)_2$, (Scharlau S.L, Spain)] and Diammonium Hydrogen Phosphate [$(NH4)_2HPO_4$, Appli-Chem, Ottowa] were used as precursors for the synthesis of CDA granules. Calcium Hydroxide (0.4 M) and Diammonium Hydrogen Phosphate (0.264 M) solutions (Ca/P molar ratio ~1.52) were prepared in deionized water separately. Later, $(NH_4)_2HPO_4$ was slowly (dropping rate ~2 mL min^{-1}) added drop wise to $Ca(OH)_2$ solution and pH was maintained at 9, followed by refluxing in a domestic microwave oven Samsung (MW101P-K) at 1000 W for 3 min. The microwave irradiated resulting reaction mixture was cooled to room temperature followed by addition of 0.4 mL of each antibiotic [Amoxicillin, Gentamicin and Chloramphenicol (Sigma-Dorset, UK)]. The suspensions were completely washed with deionized water and aged in drying oven (MW101P-K, Wise Ven, Korea) at 26 °C for 24 h. The CDA powder without antibiotics was heat treated at 1100 °C at the heating rate of 10 °C min^{-1} for 1 h. Drug release studies of the drug loaded CDA were carried out in Phosphate Buffer Saline medium (MP Biomedicals, LLC, Parc d'innovation BP 50067 Illkirch, France). 500 mg of drug loaded CDA granules were immersed in 5 mL of PBS solution. The solution was replaced with fresh medium at 1, 3, 4, 8, 16 and 24 h at 37 °C. The drug release was determined using UV–vis Spectroscopic Analysis (UV–vis Spectrophotometer Model UV 4000, O.R.I, Germany). Phosphate Buffer Saline (PBS) was used as the reference and the range of wavelength was 220–380 nm.

2.2. Characterization of control and drug loaded CDA

Structural characterization and functional groups identification was done using Fourier Transform Infrared Spectroscopy (FT-IR, Thermo Nicolet 6700, USA) with photo-acoustic cell (averaging 256 scans with 8 cm^{-1} spectral resolution). X-ray Diffraction (XRD) patterns were collected using MPD X'PERT PRO Diffractometer (PAN Analytical, The Netherlands) with monochromatic Cu-Kα, $\lambda = 0.15418$ nm. The scans were taken with a step size at 0.02° after every 1 s, in 2θ range ~20–60°. The morphological pattern of granules was observed using a Scanning Electron Microscope (SEM, JSM-6480, JEOL, Japan). The samples were mounted and sputter coated with gold prior to imaging. SEM images were collected at an activation voltage of 5 kV. Thermogravimetric Analysis and Differential Scanning Calorimetry (TGA/DSC) of the samples were carried out using TA instruments (SDT Q 600, USA). Both TGA and DSC measurements were carried out at 30–1000 °C under inert nitrogen atmosphere at a heating ramp of 10 °C min^{-1}.

3. Results and discussion

3.1. FT-IR analysis

The FT-IR spectrum of the control CDA (Fig. 1A) sample showed a characteristic peak of Hydroxyapatite; the hydroxyl band (corresponded to adsorbed water) was observed from 3600 cm^{-1} to 2800 cm^{-1} [29]. A peak at 3570 cm^{-1} was corresponded to the stretching vibration of O–H group (ν_s mode) and the bending hydroxyl peak was observed at 1640 cm^{-1} and 634 cm^{-1}. The band between 1200 cm^{-1} and 1000 cm^{-1}, centered at 1022 cm^{-1} was a characteristic of ν_3 asymmetric stretching of P–O–P in phosphate group [30,31]. The smaller shoulder peak at 960 cm^{-1} was attributed to the υ_1 symmetric stretching of P–O in PO_4^{-1} group [32]. The ν_4 and ν_3 bending vibrations of P–O peaks were observed at 539 cm^{-1} and 507 cm^{-1} respectively [31,33]. FT-IR spectra showed a significant difference in the CDA drug loaded with antibiotics and CDA alone. FT-IR spectra of the drug loaded CDA samples showed characteristic peaks corresponding to Amoxicillin, Chloramphenicol and Gentamicin functional groups. The peak at 1635 cm^{-1} was attributed to the bending vibration of N–H in Amoxicillin structure and C–N stretching peak of the amide group in Amoxicillin was appeared at 1403 cm^{-1} [34,35] as shown in Fig. 1B.

The presence of Gentamicin was confirmed by appearance of peak at 1620 cm^{-1} which was attributed to aromatic groups and the overtone peak was observed at 555 cm^{-1} (Fig. 1C). Chloramphenicol functional groups showed characteristic peaks at 1670 cm^{-1} (NCO), 1500 cm^{-1} (N–H bending peak)